CHEMICAL EXAMINATION

OF THE BARK OF

ERYTHROPHLŒUM GUINEENSE

BY

FREDERICK B. POWER, PH.D.

AND

ARTHUR H. SALWAY, PH.D., D.SC.

THE WELLCOME CHEMICAL RESEARCH LABORATORIES

FREDERICK B. POWER, PH.D., LL.D., *Director*

6, King Street, Snow Hill

LONDON, E.C.

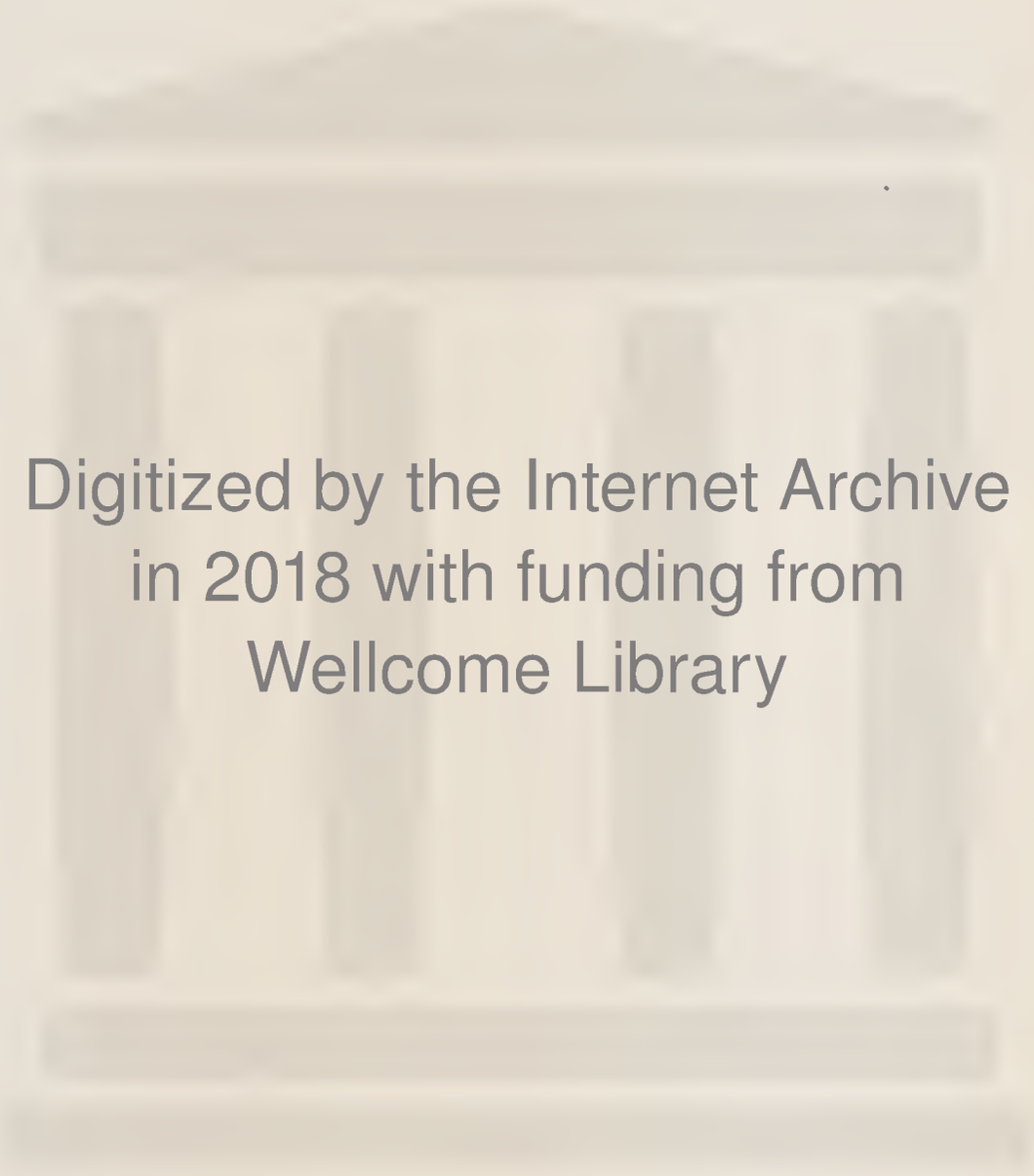

Fig. 1

ERYTHROPHLOEUM GUINEENSE, G. Don.
A felled tree, in the Belgian Congo, from which the so-called "Sassy Bark" or "Nkasa" had been collected for many years.

Fig. 2

In the foreground of this picture are to be seen a few bones, the remnants of a victim of witchcraft who had been burned after receiving a fatal dose of "Sassy Bark" or "Nkasa" (ERYTHROPHLOEUM GUINEENSE, G. Don)

Reproduced from photographs by Iner C. Wickware, Boma, Belgian Congo.

Reprinted from The American Journal of Pharmacy, August, 1912

CHEMICAL EXAMINATION OF THE BARK OF ERYTHROPHLŒUM GUINEENSE.

By Frederick B. Power and Arthur H. Salway.

A Contribution from the Wellcome Chemical Research Laboratories, London.

The genus *Erythrophlœum* appears to comprise but few species, of which the best known is *E. Guineense,* G. Don of Central and Western Africa. Other species are *E. Couminga,* Baill. from Madagascar; *E. Fordii,* Oliv. from Farther India; *E. chlorostachys* (F. von Mueller), Baill. from Australia, and *E. densiflorum* (Elmer), Merrill, formerly known as *Cynometra densiflora,* Elmer, from the Philippines.[1] In addition to these, Planchon [2] notes that Gagnepain (*Notulæ systematicæ* de Lecomte. Herbier du Muséum de Paris, *t.* ii, fasicule 4, p. 111 *et seq.*) has recently named and described two species which are peculiar to Farther India, namely: *E. cambodanium* and *E. succirubrum.*

The bark of *Erythrophlœum Guineense,* G. Don (Nat. Ord. *Leguminosæ*) is known by a number of common names, such as "sassy bark," "mancona bark," "red-water tree bark," "casca bark," "doom bark," and in the vernacular of the Congo as "Nkasa." [3] It was brought to notice many years ago [4] on account

[1] *Philippine Journal of Science,* 1909, 4, 267.

[2] *Annales du Musée colonial de Marseille,* 1911, 19, 6.

[3] The designation "Nkasa" does not seem to be a specific one, since it is also applied to *Strychnos Dewevrei,* Gilg, and possibly to other plants. It has, furthermore, been stated that the expression "Nkasa" does not denote, as has been supposed, the plant from which the ordeal poison has been obtained, but the ordeal itself, and that it is used throughout the Congo ("Compagnie du Kasai. Mission permanente d'Études scientifiques." Résultats de ses Recherches botaniques et agronomiques, mis en order et annotés par E. de Wildeman, Brussels, 1910, p. 211 *et seq.*).

[4] This Journal, 1849, 21, 97; 1851, 23, 301, and 1852, 24, 195. Also *Pharm. Journ.,* 1856, 16, pp. 233, 373.

of its intensely poisonous properties, by reason of which it has long been employed by the natives of Western Africa as an ordeal in their trials for witchcraft and sorcery, as well as for other criminal purposes. It also apparently enters into the composition of the arrow poison of the Pigmies.[5] A description of the bark, together with observations respecting its physiological action or that of its constituent alkaloid, has been recorded in various works of reference, such as the "Dispensatories" and other commentaries on the Pharmacopœias, as well as in the periodical literature of pharmacy and medicine. Since these sources of information are so readily available, the present consideration of the literature may chiefly be restricted to some previously published statements respecting the alkaloidal constituent of the bark.

The so-called "Sassy Bark" appears to have been first chemically examined by Gallois and Hardy,[6] who isolated from it a very toxic alkaloid which they designated erythrophleine. This was stated to have been obtained by one method as a transparent, amorphous solid, of a pale amber color, firm consistence, and gummy to the touch, whereas by another method it was yellowish-white, transparent, and had a crystalline appearance, which could be confirmed by the microscope. It was soluble in water and in alcohol, but very slightly soluble in ether or chloroform. The alkaloid was furthermore described as yielding a crystalline hydrochloride and platinichloride, and as giving with potassium permanganate and sulphuric acid a violet color, less intense than that produced by strychnine, and soon changing to a dirty brown. No analysis, melting point, or other specific characters of any of these substances were, however, recorded. The same authors state to have obtained from *Erythrophlœum Couminga* an alkaloid which was believed to be closely related to erythrophleine, if not identical with it.

A pharmacological investigation of erythrophleine was subsequently conducted by Harnack and Zabrocki.[7] The alkaloid employed by them was prepared by a German manufacturer, and it was recorded that the base, as well as its salts and double salts,

---

[5] *Pharm. Journ.*, 1891, [iii], **21**, 917.

[6] *Journ. Pharm. Chim.*, 1876, **24**, 25, and *Bull. Soc. Chim.*, 1876, **26**, 39.

[7] *Arch. f. Exp. Path. Pharm.*, 1882, **15**, 404.

could be obtained only in the form of clear syrups. The substance produced both with cold-blooded and warm-blooded animals the combined effects of digitalin and picrotoxin (clonic spasms). After an interval of several years the investigation was renewed by Harnack,[8] with the use of material which had been procured from the same manufacturer as that previously employed. This specimen of erythrophleine hydrochloride was, however, in the form of a fine, pale yellow, amorphous powder, which, on keeping, agglomerated to a dry, solid mass. In distinction from the alkaloid previously examined, it produced only a digitalin-like effect, and not also that of picrotoxin. The base obtained from this salt was almost completely insoluble in water, but readily soluble in alcohol and in ether, and, contrary to the statement of Gallois and Hardy (*loc. cit.*), showed no tendency to crystallize. From analyses of the platinum salt, the base was considered to possess the formula $C_{28}H_{43}O_7N$ or $C_{28}H_{45}O_7N$, but the difficulty was noted of obtaining preparations which were sufficiently uniform in composition to ensure trustworthy results. On heating with concentrated hydrochloric acid it yielded an amorphous, acidic product, designated erythrophleic acid, $C_{27}H_{40}O_8$ or $C_{27}H_{42}O_8$, and methylamine, $CH_3.NH_2$, whereas the alkaloid first examined, when similarly treated, gave apparently the same acidic substance and a base resembling pyridine, which was termed manconine.

It would seem probable that the varying results obtained in the above-mentioned investigations may be attributed, as Harnack (*loc. cit.*, p. 562) and others have suggested, to the use of barks from different species of *Erythrophlœum*, or possibly from varieties of the same species. This view has recently been corroborated by observations personally communicated to us by Mr. Iner C. Wickware, to whom we are indebted for the material employed in the present investigation and also for photographs from which the illustrations accompanying this paper were produced. The material supplied by Mr. Wickware was designated by him as "Nkasa Bark," but its identity with the so-called "Sassy Bark" (from *Erythrophlœum Guineense*, G. Don) was kindly confirmed by Mr. E. M. Holmes, F.L.S.

The extended use of "Nkasa" bark in the Congo for criminal

[8] *Arch. Pharm.*, 1896, 234, 561.

purposes, and the great suffering thereby produced, which had been witnessed by Mr. Wickware, led him to bring the bark to our notice, as it was hoped that a study of its constituents would render it possible to suggest a means of obviating the destructive effects of the poison. Inasmuch as the alkaloid erythrophleine had hitherto not been obtained in a form which permitted of its more exact characterization, and the statements concerning it being also somewhat at variance, it was deemed desirable to subject the "Nkasa" bark to a further chemical examination. The results which have now been obtained are summarized at the end of this paper.

EXPERIMENTAL.

The material employed for this investigation was obtained from the Belgian Congo, West Africa, through the kindness of Mr. Iner C. Wickware, of the Christian Missionary Alliance, at Boma, to whom our best thanks may here be expressed. It was collected from living trees, and, as already noted, agreed in its characters with the recorded descriptions of the bark of *Erythrophlœum Guineense,* G. Don.

A small portion (10 grammes) of the ground bark was tested for the presence of an alkaloid with a positive result.

For the purpose of a complete chemical examination 72.9 kilogrammes of the coarsely ground bark were completely extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, 27.68 kilogrammes of a dark red, viscid extract were obtained.

A quantity (4.5 kilogrammes) of the above-mentioned extract was mixed with water and distilled in a current of steam, but it yielded no essential oil. After this operation there remained in the distillation flask a deep red, aqueous liquid (A) and a quantity of a viscid resin (B), which solidified on cooling. The resin was thoroughly washed with water, and the washings added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid was concentrated under diminished pressure, and then repeatedly extracted with ether. The ethereal liquid was washed, dried, and the solvent removed, when 12 grammes of a brown, varnish-like solid were obtained.

Isolation of Luteolin, $C_{15}H_{10}O_6$.

The above-mentioned ethereal extract was redissolved in ether, and the solution shaken first with aqueous ammonium carbonate, which, however, extracted but a small amount of amorphous, gummy material. It was then shaken with successive portions of aqueous sodium carbonate until nothing further was removed by this reagent. The sodium carbonate extracts were united and acidified, when a lemon-yellow, crystalline solid was precipitated. This was collected and recrystallized several times from dilute alcohol, when it was obtained in pale yellow, glistening needles, which melted and decomposed at 323°. The amount of pure substance thus isolated from 4.5 kilogrammes of the original alcoholic extract was 0.2 gramme, and therefore represented 0.0017 per cent. of the weight of the bark. On subsequently treating 12 kilogrammes of the extract in the manner already described, a further quantity (0.5 gramme) of the yellow, crystalline substance was obtained.

0.1012 * gave 0.2338 CO_2 and 0.0350 H_2O. C = 63.0; H = 3.8.
0.0898 * gave 0.2072 CO_2 and 0.0312 H_2O. C = 62.9; H = 3.9.
$C_{15}H_{10}O_6$ requires C = 62.9; H = 3.5 per cent.

The substance was readily soluble in aqueous sodium carbonate and sodium hydroxide, forming deep yellow liquids, whilst its alcoholic solution gave with ferric chloride an intense green color. It dissolved in concentrated sulphuric acid, yielding a yellow solution which gradually develops a green fluorescence. On heating the substance for some time with acetic anhydride, and subsequently removing the greater portion of the latter, a compound separated, which, when recrystallized from ethyl acetate, was obtained in fine, colorless needles, melting at 222°–224°. This compound was likewise analyzed.

0.1038 gave 0.2320 CO_2 and 0.0384 H_2O. C = 60.9; H = 4.1.
$C_{15}H_6O_6(CO.CH_3)_4$ requires C = 60.8; H = 4.0 per cent.

From the above results it may be concluded that the yellow substance possesses the empirical formula $C_{15}H_{10}O_6$, and that it contains four hydroxyl groups. These facts, together with the above-described characters, render it evident that the substance is

* Dried at 120°.

luteolin, a tetrahydroxyflavone, which was first isolated from the so-called Dyer's Weed (*Reseda luteola,* Linné), and which also occurs in the leaves of *Digitalis purpurea,* Linné, being identical with the compound termed digitoflavone.[9]

The ethereal liquid from which the luteolin had been removed by extraction with sodium carbonate, as above described, was next shaken with a solution of sodium hydroxide, which, however, removed practically nothing. On finally drying and evaporating the ethereal liquid, it yielded about 2 grammes of amorphous material, from which nothing definite could be obtained.

The deep red aqueous liquid which had been completely extracted with ether was subsequently shaken with successive portions of warm amyl alcohol, these extracts being then united, washed with a little water, and concentrated to a small volume under diminished pressure. The concentrated amyl alcohol extracts deposited nothing on keeping, but on the addition of toluene an amorphous, nearly colorless precipitate was formed, which, when collected and dried on a porous tile, amounted to 25 grammes. This product was extremely soluble in water, giving a deep red solution, from which sodium carbonate precipitated a brown, amorphous solid, soluble in an excess of the reagent. The solid substance was found to contain traces of nitrogen, but nothing definite could be isolated from it. In order to ascertain whether it was glucosidic in character, a portion was heated for some time with 5 per cent. sulphuric acid. During this operation a large amount of resinous matter separated, which was subsequently removed by filtration, and the clear, acid liquid then extracted with ether. The ethereal liquid yielded, besides some amorphous material, a very small amount of a crystalline substance, which was identified by its melting point and reactions as gallic acid. The aqueous, acid liquid was finally deprived of sulphuric acid by means of baryta, concentrated, and examined for sugar, but with a negative result. It was therefore evident that the above-described solid was not glucosidic.

The original aqueous liquid (A), after having been extracted with ether and amyl alcohol as described above, still retained a deep red color, and gave slight precipitates with the usual alkaloid reagents. A small portion of the liquid was treated with basic lead acetate, which produced an abundant, dark colored precipitate, and

---

[9] *Ber. d. deutsch. chem. Ges.,* 1899, **32,** 1184.

the mixture filtered. The filtrate was deprived of lead by means of hydrogen sulphide, again filtered, and concentrated to a small volume. It was found to contain a considerable quantity of sugar, which yielded an osazone melting and decomposing at 210°. The basic lead acetate precipitate was suspended in water, decomposed by hydrogen sulphide, and the mixture filtered. The resulting liquid evidently contained a large amount of tannin, and, although it gave precipitates with the alkaloid reagents and with sodium carbonate, nothing definite could be isolated from it. There was no indication of the presence of a glucoside.

The main portion of the aqueous liquid was carefully neutralized with sodium carbonate, when a brown, amorphous precipitate was deposited, which, after being collected, washed with water, and dried, amounted to about 15 grammes. This product contained nitrogen, but it was very indefinite in character. It was insoluble in water and dilute mineral acids, as also in ether or chloroform, and was only sparingly soluble in alcohol. It dissolved readily in alkalies, giving a deep brown solution. When warmed with dilute hydrochloric acid, the liquid gave no reaction with alkaloid reagents. All attempts to prepare derivatives from it led only to the formation of resinous substances.

Isolation of the Alkaloid, Erythrophleine.

The aqueous liquid from which the above-mentioned brown, amorphous product had been separated, was made strongly alkaline with sodium carbonate and repeatedly extracted with ether. These extracts were united, washed with a little water, then dried, and a current of dry hydrogen chloride passed into the ethereal liquid. The alkaloid present was thus precipitated as a viscid hydrochloride, from which the ether was removed by decantation. The hydrochloride, amounting to about 1 gramme, was dissolved in a little alcohol, and ethyl acetate added, but no precipitation ensued. On subsequently evaporating the liquid to dryness in a vacuum desiccator over sulphuric acid, the salt was obtained in the form of a brown, scaly solid, which was soluble in water and responded to the tests for an alkaloid with the usual reagents. In order to further purify this product, it was redissolved in water, and aqueous sodium carbonate cautiously added until the precipitation of the base was complete. The mixture was then extracted with ether, filtered to remove some insoluble resinous matter, and the ethereal

liquid again treated with dry hydrogen chloride. The gummy hydrochloride thus obtained was dissolved in ethyl acetate containing some alcohol, and the solution then evaporated at the ordinary temperature under diminished pressure over sulphuric acid. In this manner the alkaloidal salt was obtained as an almost colorless, amorphous solid, amounting to 0.6 gramme. It was readily soluble in water or alcohol, and was extremely hygroscopic, becoming gradually converted into a brown, transparent, glutinous mass. The free base, which has been termed erythrophleine by previous investigators, was a colorless, amorphous solid, readily soluble in ether, ethyl acetate, or alcohol, but insoluble in water. It yielded a bright yellow picrate, and an almost colorless gold salt, but neither of these derivatives could be obtained in a crystalline state.

The properties of erythrophleine, as described above, do not entirely agree with those recorded by Gallois and Hardy (*loc. cit.*), but are more in accordance with the later observations of Harnack.[10] A commercial specimen of erythrophleine hydrochloride, which was obtained by us from the same source as the product last employed by Harnack, was also similar in character to that described by him. It was a nearly colorless, amorphous powder, which on keeping in a closed tube gradually became converted into a brown, gummy mass. It dissolved readily in water, yielding a colorless solution, which was neutral to litmus and responded to the usual alkaloidal tests. On the addition of aqueous sodium carbonate the free base was precipitated as a gummy solid, which was insoluble in water or alkalies, but readily soluble in ether. The dry ethereal solution of the base, when treated with dry hydrogen chloride, yielded the hydrochloride in the form of a viscid oil and not in the original condition as an amorphous solid. Both the picrate and the aurichloride of the base were likewise amorphous. The amount of chlorine in this commercial specimen of erythrophleine hydrochloride was determined with the following result:

0.1652 gave 0.0462 AgCl. Cl = 6.9.
$C_{28}H_{43}O_7N.HCl$ requires Cl = 6.6 per cent.

Although the result of this determination is in approximate agreement with the formula assigned to erythrophleine by Harnack,

---

[10] *Arch. Pharm.*, 1896, **234**, 561.

it is evident that the substance is of too indefinite a character to permit of any deduction respecting its ultimate composition.

The aqueous liquid from which the erythrophleine had been removed, as above described, was made strongly alkaline with sodium hydroxide and extracted with various solvents, but no further quantity of alkaloidal substance was thus obtained. The proportion of alkaloid in the drug was therefore extremely small, since the amount of hydrochloride originally obtained (about 1 gramme) represented not more than 0.008 per cent. of the weight of bark employed.

Examination of the Resin (B).

The resinous material obtained from the original alcoholic extract of the bark, as previously described, formed, when dry, a dark brown, brittle mass, which could be reduced to a powder. It amounted to 1600 grammes, and thus represented 13.5 per cent. of the weight of the bark. For the purpose of its examination, 500 grammes of the resin were dissolved in alcohol, the solution mixed with purified sawdust, and the thoroughly dried mixture successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extracts, dried at 100°, were obtained:

Petroleum (b. p. 30–45°)	extracted	35.5	grammes	= 7.1	per cent.
Ether	"	26.0	"	= 5.2	" "
Chloroform	"	3.5	"	= 0.7	" "
Ethyl Acetate	"	26.0	"	= 5.2	" "
Alcohol	"	400.0	"	= 80.0	" "
		Total 491.0	grammes	= 98.2	per cent.

Petroleum Extract of the Resin.

This extract was a dark brown, soft, fatty solid. It was tested for the presence of an alkaloid, but with a negative result. The whole of the extract was then hydrolyzed by heating for a short time with an alcoholic solution of potassium hydroxide, after which the alcohol was removed, water added, and the alkaline mixture repeatedly extracted with ether. The ethereal extracts were united, washed, dried, and the solvent removed, when a quantity (20 grammes) of a reddish-brown, viscid solid was obtained.

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The above-mentioned unsaponifiable material was distilled under diminished pressure, when a small quantity passed over below 260°/10 mm., but the greater portion distilled with slight decomposition above 300°/10 mm., a small final fraction being separately collected. The principal portion did not crystallize on cooling, but formed a viscid, transparent, yellow gum. A little of this substance, when dissolved in a mixture of chloroform and acetic anhydride, and a drop of sulphuric acid subsequently added, gave a reddish-brown coloration, rapidly changing to green, thus indicating the presence of a phytosterol. The gummy substance was therefore dissolved in hot alcohol, and the liquid cooled with vigorous agitation, when a colorless solid was gradually deposited. This was collected and recrystallized from alcohol, from which it separated in colorless, glistening leaflets, melting at 130–133°.

0.2378, on heating at 110°, lost 0.0112 H_2O. $H_2O = 4.7$.
0.1334* gave 0.4110 CO_2 and 0.1459 H_2O. $C = 84.0$; $H = 12.1$.

$C_{27}H_{46}O$, H_2O requires $H_2O = 4.5$ per cent.
$C_{27}H_{46}O$ requires $C = 83.9$; $H = 11.9$ per cent.

The above-described substance was evidently a phytosterol, and it gave the color reactions of that class of compounds.

The small final fraction obtained by the distillation of the unsaponifiable material yielded a phytosterol which crystallized in leaflets melting somewhat indefinitely between 135° and 142°. It would thus appear that more than one phytosterol was present in the distilled product, but the amount of substance was too small to permit of a more complete separation.

Examination of the Fatty Acids.

Isolation of Cerotic Acid, $C_{26}H_{52}O_2$.

The alkaline, aqueous solution of potassium salts, which had been extracted with ether as above described, was acidified with dilute sulphuric acid, and the precipitated fatty acids dissolved in ether. The ethereal solution was washed, dried, and the solvent removed, when a semi-solid residue was obtained, amounting to 12

* Anhydrous substance.

grammes. This was distilled under diminished pressure, and two fractions collected, which passed over at 200–250°/12 mm., and above 250°/12 mm., respectively. The latter fraction rapidly solidified, and was purified by crystallization from ethyl acetate, when an acid was obtained which separated in minute, colorless needles, melting at 76–77°.

0.0657 gave 0.1895 CO_2 and 0.0796 H_2O. C = 78.7; H = 13.4.
$C_{26}H_{52}O_2$ requires C = 78.8; H = 13.1 per cent.

This substance was thus identified as cerotic acid.

The fraction of fatty acids distilling at 200–250°/12 mm. amounted to 6 grammes, and was evidently a mixture of saturated and unsaturated acids. It was therefore converted into the lead salt, and the latter treated with ether. The portion of lead salt which was insoluble in that solvent yielded the saturated acids, which, after recrystallization from alcohol, melted at 48–50°.

0.4208 required for neutralization 15.7 c.c. $\frac{N}{10}$ KOH. N.V. = 209.3.
$C_{16}H_{32}O_2$ requires neutralization value = 219.1.
$C_{18}H_{36}O_2$ requires neutralization value = 197.5.

It is thus evident that the saturated acids present in the above-mentioned fraction consisted of a mixture of palmitic and stearic acids, and apparently in about equal proportions.

The portion of lead salt which was soluble in ether yielded 3.5 grammes of unsaturated acids, which distilled at 215–225°/10 mm. These were analyzed, and the neutralization and iodine values determined, with the following results:

0.1753 gave 0.4950 CO_2 and 0.1836 H_2O. C = 77.0; H = 11.6.
0.5902 required for neutralization 20.2 c.c. $\frac{N}{10}$ KOH. N.V. = 192.0.
0.1096 absorbed 0.1449 iodine. Iodine value = 132.2.
$C_{18}H_{34}O_2$ requires C = 76.6; H = 12.1 per cent.
Neutralization value = 198.9; Iodine value = 90.1.
$C_{18}H_{32}O_2$ requires C = 77.1; H = 11.4 per cent.
Neutralization value = 200.4; Iodine value = 181.4.

It would appear from these results that the unsaturated acids consisted of a mixture of oleic and linolic acids.

Ether Extract of the Resin.

This extract was a brown, brittle solid, amounting to 26 grammes. It was digested with ether, when the greater portion passed into solution, while a small amount of a colorless powder remained undissolved. The latter was very sparingly soluble in alcohol, and when dissolved in chloroform, a little acetic anhydride added, and subsequently a drop of concentrated sulphuric acid, it gave a pink coloration, rapidly changing to blue and green. It also yielded an acetyl derivative, which separated in colorless leaflets, melting at 162–163°. From these characters it may be concluded that the sparingly soluble substance was the dihydric alcohol, ipuranol, $C_{23}H_{38}O_2(OH)_2$, but the amount obtained was not sufficient for analysis.

The above-mentioned ethereal liquid was shaken successively with dilute hydrochloric acid and aqueous solutions of ammonium carbonate, sodium carbonate, and sodium hydroxide. The hydrochloric acid removed only traces of a gummy base, which gave reactions with the usual alkaloid reagents, whilst the ammonium carbonate caused the precipitation of a small amount of a resinous solid of indefinite character. The sodium carbonate extract yielded, on acidification, a pale yellow solid. This was collected, and crystallized from dilute alcohol, when it separated in pale yellow needles, and gave a colorless acetyl derivative melting at 222–224°. It was thus found to be identical with luteolin, which had previously been isolated, as above described.

The final extraction of the ethereal liquid with sodium hydroxide yielded nothing but amorphous products, and on subsequently evaporating the ether only traces of a soft resin remained.

Chloroform, Ethyl Acetate and Alcohol Extracts of the Resin.

The chloroform extract of the resin was a dark brown, viscid solid, amounting to only 3.5 grammes, and nothing was isolated from it.

The ethyl acetate extract of the resin was a brown, viscid solid, which amounted to 26 grammes. In order to ascertain whether it contained anything glucosidic, it was heated for some time with a 5 per cent. solution of sulphuric acid in aqueous alcohol. On subsequently distilling the mixture with steam, no volatile oil or

acid passed over. The distillation flask then contained, besides the aqueous liquid, a quantity of a viscid resin, which was collected, dissolved in alcohol, dried on purified sawdust, and the mixture extracted with ether in a Soxhlet apparatus. From this ethereal extract a small amount of a yellow, crystalline substance was obtained which proved to be identical with the previously described luteolin.

The aqueous, acid liquid, from which the above-mentioned viscid resin had been separated, was first extracted with ether, which, however, removed only a small quantity of gummy material. It was then freed from sulphuric acid by means of barium hydroxide, and finally concentrated under diminished pressure to a small volume. This liquid readily reduced Fehling's solution, and yielded an osazone melting and decomposing at 210°.

The results above described indicated the presence of a glucoside in the ethyl acetate extract of the resin, and it is probable that the small amount of luteolin which was isolated after the treatment with dilute sulphuric acid represented one of its hydrolytic products.

The alcohol extract of the resin was a brownish-black, brittle solid, which amounted to 400 grammes, and represented 80 per cent. of the total resinous material employed. It was completely amorphous, and as it appeared to undergo no change when heated with dilute sulphuric acid in aqueous alcohol, it evidently contained nothing of a glucosidic character.

Physiological Tests.

In order to determine the action of the extract prepared from *Erythrophlœum* bark, and of some of the products obtained therefrom, a number of tests were kindly conducted for us by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories, to whom our best thanks may here be expressed.

One gramme of the original alcoholic extract, representing about 2.6 grammes of the bark, was administered to a dog, when it caused continuous vomiting and a marked slowing of the heart-beat. One gramme of the total resinous material (= 7.4 grammes of bark) had much less effect, but caused some vomiting. A quantity of the water-soluble portion of the original alcoholic extract, representing 4 grammes of the bark, caused retching, without actual vomiting, and a distinct action on the heart.

The previously described amyl-alcohol extract of the aqueous liquid (A) was tested by dissolving 0.07 gramme of the respective extract in water, and injecting this intravenously into a rabbit, but it had no perceptible effect. The amount of substance employed represented about 13.6 grammes of the bark.

The brown, amorphous substance which was precipitated from the aqueous liquid (A) by its neutralization with sodium carbonate, as previously described, could not be tested by intravenous injection on account of its insolubility. A small quantity (0.25 gramme) of the product, representing about 216 grammes of the bark, was therefore given to a dog by the mouth, but no perceptible effect was produced.

The alkaloid erythrophleine, which was tested in the form of its hydrochloride, produced results similar to those obtained from a commercial specimen of this salt, which was examined for the purpose of comparison, and in both cases the action was such as has been described by previous observers. When given intravenously to a rabbit, 0.001 gramme of commercial erythrophleine hydrochloride caused marked slowing of the heart-beat, whereas with 0.002 gramme the same effect was followed by secondary quickening of the heart-beat, paresis, trismus, and finally death in convulsions in about half an hour. Similar results were obtained from the alkaloid isolated in the course of the present investigation, and 0.005 gramme of the hydrochloride of this base killed a small rabbit in less than five minutes. Both preparations of the alkaloid, when injected intravenously into pithed cats in doses of 0.0025 gramme, caused quite typical digitalis-like effects, the rise of blood pressure being large, and the heart passing through delirium to permanent systole.

Since the precipitate obtained by treating a small quantity of the water-soluble portion of the original alcoholic extract with basic lead acetate, when suspended in water and decomposed by hydrogen sulphide, yielded a liquid which possessed in a marked degree the characteristic erythrophleine action, it would appear that an appreciable amount of active substance had been precipitated by the lead salt. As already noted, however, nothing definite could be obtained from the lead compound, and for the purpose of isolating the alkaloid from the main portion of the original aqueous liquid (A) the latter was not subjected to treatment with basic lead acetate.

Summary.

The results of the present investigation of the bark of *Erythrophlœum Guineense,* G. Don, and the deductions therefrom, may briefly be summarized as follows:

A quantity of the bark was completely extracted with hot alcohol, and the resulting concentrated extract distilled in a current of steam, but it yielded no essential oil.

From the portion of the extract which was soluble in water the following substances were isolated: a very small amount of luteolin, $C_{15}H_{10}O_6$, and a small amount of an alkaloid which agreed in its characters and physiological action with erythrophleine, as described by Harnack (*Arch. Pharm.,* 1896, **234,** 561). Neither the alkaloid nor its salts could be obtained in a crystalline state, and they were therefore not considered suitable for analysis. The aqueous liquid contained, furthermore, besides some indefinite amorphous material, a considerable quantity of tannin, and a sugar which yielded *d*-phenylglucosazone, melting at 210°.

The portion of the alcoholic extract which was insoluble in water consisted of a dark brown, brittle resin, and represented 13.5 per cent. of the weight of the bark. From this product the following substances were obtained: a phytosterol, $C_{27}H_{46}O$ (m. p. 130–133°); cerotic, stearic, palmitic, oleic and linolic acids; and very small amounts of ipuranol, $C_{23}H_{38}O_2(OH)_2$, and luteolin, $C_{15}H_{10}O_6$. A portion of the latter compound was apparently contained in the resin in the form of a glucoside.

Inasmuch as the results of a preliminary test had indicated a much larger proportion of alkaloid to be contained in the bark than could subsequently be isolated, it appears probable that some change had taken place during the processes of extraction. This could not be more precisely determined on account of the very indefinite character of the base, which also precluded its further chemical study.

Since the bark under consideration is an exceedingly violent poison, and is largely used in West Africa for criminal purposes, it may finally be noted that the recognized and apparently most efficient antidote consists in the prompt administration of an emetic, or use of the stomach-pump, with subsequent stimulant remedies.